SERVIZIO SANITARIO REGIONALE
EMILIA - ROMAGNA
Istituto Ortopedico Rizzoli di Bologna
Istituto di Ricovero e Cura a Carattere Scientifico

"Global collaboRAtion on CIC-DUX4, BCOR-CCN3, high-grade Undifferentiated round cell sarcoma (URCS) project'

Study code	**GRACefUl**
Sponsor's Name and Address:	IRCCS Istituto Ortopedico Rizzoli Via di Barbiano 1/10 40136 Bologna Italy
Study Number/Version/ Date:	Vers 2.0 29 July 2021
Coordinating Center:	IRCCS Istituto Ortopedico Rizzoli SC Osteoncologia sarcomi dell'osso e dei tessuti molli e terapie innovative Via Pupilli 1 40136 Bologna, Italy
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Methodology:	Experimental study with biological material : case series review of clinical and histological data
Type:	Academic
Founding:	None

Protocol Signature

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Study Title: "Global collaboRAtion on CIC-DUX4, BCOR-CCN3, high-grade Undifferentiated round cell sarcoma (URCS) project'

Study Acronym/Code: GRaCefUl

Protocol Version and Date: Version 2.0 of 29 July 2021

I read this protocol and I accept to conduct this trial in accordance with the protocol stipulations, GCP guidelines and the Declaration of Helsinki.

Study global coordinator :

Dott. ssa Emanuela Palmerini
SC Osteoncologia sarcomi dell'osso e dei tessuti molli
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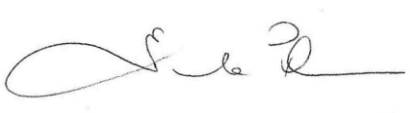
Local Site Name: IRCCS Istituto Ortopedico Rizzoli

Local Site Principal Investigator Name: Dr. Emanuela Palmerini

Local Site Principal Investigator Signature	Signature Date
Emanuela Palmerini	29 July 2021



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BACKGROUND

Undifferentiated round cell sarcomas (URCSs) represent a diagnostic challenge. URCSs cell histology and expression of CD99 mostly resemble Ewing sarcoma. However, they also may include other tumors such as mesenchymal chondrosarcoma, synovial sarcoma, or small cell osteosarcoma. Definitive classification usually requires detection of entity-specific mutations.

Recently, the use of modern molecular techniques led to the description of a variety of new, rare, sarcoma subtypes, which have morphological appearance similar to Ewing sarcomas ("Ewing-like" sarcomas), but carry gene fusions different from that of Ewing sarcoma, with CIC-DUX4, and BCOR-CCNB3 being the most frequent. Other non-Ewing round cells sarcomas are characterized by rearragement of CIC (CIC-rearraged sarcomas) or BCOR (BCOR-rearraged sarcomas) with other genes, by Internal Tandem Duplication of BCOR or by other translocations (i.e.: CRTC1-SS18).

The identification of these gene fusions suggests that other, yet to be identified, gene fusions could be associated with this type of tumour. Furthermore, novel gene fusions in Ewing-like" sarcomas have been reported recently, in case reports, including CIC-FOXO4 BCOR-MAML3 and ZC3H7B-BCOR. Identifying these genetically defined entities may contribute towards understanding the pathogenesis and the behaviour of these tumours. The optimal treatment for these ultra rare tumors is not known.

We propose to collect all cases of URCSs treated within the referral centres for sarcoma, including Italian sarcoma group network (ISG), in order to reclassify and describe their treatment and outcome.

STUDY DESIGN

This is a multicenter retrospective and prospective study that will analyze all cases with a diagnosis of URCSs from 01 January 1983 and all new cases referring to each participant site.

OBJECTIVE OF THE STUDY

Primary Objective

Correlate clinical and pathological variables with event-free survival (EFS) and overall-survival (OS) in URCSCs

Secondary objective

Molecular re-classification of URCSs based on presence of CIC-DUX4 and BCOR-CCNB3 fusion genes, detected by FISH and/or PCR

Exploratory objective

In all the "true-negative" URCSs where NGS and RNA-seq data is already available, genomic/genetic characteristics will be correlated with clinical outcome

POPULATION

Inclusion criteria

1. Patients with URCSs treated from 01 January 1983 and all new patients will be included. The study will include the following:
   - URCSs negative for all Ewing markers, CIC-DUX4 and BCOR-CCNB3 ("true-negative")
   - URCSs CIC –DUX4 fusion-positive, or
   - URCSs BCOR-CCNB fusion-positive sarcoma;
2. Clinical and outcome information available.
3. Histological slides/formalin-fixed paraffin-embedded tissue tumor (FFPE) blocks from archive available to perform the histology analysis/frozen tissue representative of the tumor available.

Exclusion criteria

1) Diagnosis different from URCSs
2) Patient with no clinical or outcome information available

The study will include male and female patients without limit of age

MATERIAL AND METHODS

Patients with a morphological diagnosis of URCSs will be included and will be classified as

- URCSs negative for all Ewing markers, CIC-DUX4 and BCOR-CCNB3 ("true-negative") or,
- URCSs CIC –DUX4 fusion-positive, or
- URCSs BCOR-CCNB fusion-positive sarcoma;

Formalin-fixed paraffin embedded tissue and/or frozen tumoral tissue of URCSs cases *negative* for all Ewing's sarcoma molecular fusions, and CIC-DUX4 and/or BCOR-CCNB3 status unknown, will be

analyzed for the presence of CIC-DUX4 / BCOR-CCNB3 translocations fusion at each Institution. All these molecular analysis will be performed by using real-time PCR and/or FISH analysis.
Positive cases will be then re-classified accordingly, while negative cases will be defined as "true-negative". For patients defined as "true-negative, if NGS and RNA-seq data are already available, genomic/genetic characteristics will be collected.
Treatment, outcome, and prognostic factors will be related with the molecular re-classification.
Each participating center will have to send to the global study coordinator the *pathology form* correctly filled in.

STATISTICS

For survival analysis the following factors will be correlated with Overall Suvival (OS) and Event Free Survival (EFS): stage (localised vs metastatic), histology (CIC-DUX4, BCOR-CCNB3, other), age (pediatric < 18 years vs adult ≥ 18 years), gender, pathologic response (good vs poor) to neoadjuvant chemotherapy.
EFS and OS will be estimated according to the Kaplan and Meier method with their respective 95% confidence intervals (CI) and calculated from the first day of chemotherapy administration to death or last follow-up visit. A Cox regression model will also be used to examine the effect of factors on OS and EFS.
For retrospective analysis, it is expected to include about 200 patients. For prospective study, we expect to include 60 patients.

ENROLLMENT PROCEDURE

Patients considered eligible and who have provided a written informed consent will be included into the study and their material will be used for the purpose of this research according to the protocol and to the informed consent provided.
For the retrospective analysis, the investigator will extract from Institute database the list of potential eligible patients for whom the tumor material is available. After the verification of the presence of the study enrollment criteria, the patient will be included in the study.
Due to the high incidence of mortality of the disease under investigation, it would be possible that some eligible subjects will be deceased.

DATA COLLECTION

Clinical data will be retrieved by reviewing clinical charts, radiology and pathology report by local investigators.

A protocol-specific CRF reporting pathologic report at the time of study inclusion and the results of re-classification will be provided.

A CRF is required and should be completed for each included subject.

ETHICS AND QUALITY ASSURANCE

The clinical trial protocol and its documents will be sent before initiating the study to the competent Authorities and Ethics Committees of each participating country for its approval.

The responsible investigator will ensure that this study is conducted in agreement with either the most updated Declaration of Helsinki and all the international and local laws that apply to clinical trials and to patient protection.

The protocol has been written, and the study will be conducted according to the principles of the ICH Harmonized Tripartite Guideline for Good Clinical Practice
(ref: http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).

INFORMED CONSENT

All patients will be informed, by the investigator, of the aims of the study, the possible risks and benefits that will derive from the study participation.

The Investigator must clearly inform that the patient is free to refuse participation in the study and that can withdraw consent at any time and for any reason.

They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

The informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

The Investigator must also sign the Informed Consent form, and will keep the original at the site and a copy of the original must be handed to the patient.

The competent ethics committee for each Institution participating to the study must validate local informed consent documents before the study can be opened. It will be emphasized that the

participation is voluntary and that the patient is allowed to refuse further participation in the study whenever he/she wants. This will not prejudice the patient's subsequent care.

For retrospective analysis, due to the high incidence of mortality of the disease under investigation, it would be possible that some potential eligible subjects will be deceased.

CONFIDENTIALITY

In order to ensure confidentiality of clinical trial data as disposed the national and European applicable regulation, data will be only accessible for the trial Sponsor and its designees, for monitoring/auditing procedures, the Investigator and collaborators, the Ethics Committee of each corresponding site and the Health Authority.

Investigator and the Institution will allow access to data and source documentation for monitoring, auditing, Ethic Committee revision and inspections of Health Authority, but will have to maintain at all times personal data confidentiality as specified in all the applicable regulations.

All patients included in the study will be identified with a numeric code. Study Responsible and Collaborators of each laboratory and participating Institutions will have to keep a patients' study inclusion registry containing the following personal data of each patient: name, surname, address and corresponding identification code used for this study.

Study Responsible, Investigators and Collaborators of each laboratory and participating Institutions will guarantee that patients anonymity will be kept at all times and that their identity will be protected from access from unauthorized persons and Institutions.

PUBBLICATION OF RESULTS

The results from this study can be published or shown at scientific conferences. According to usual practice, this multicenter study will be published as a whole, and not with the data obtained separately from each of the sites participants. It is expected that other articles are published about the exploratory aspects of this trial once the main data has been published. The final publication of the trial results will be written by the Coordinator Investigator.

All publications (papers, abstracts, presentations…) including data from the present trial will be submitted for review to all co-authors prior to submission.

SPONSOR ROLE AND RESPONSIBILITY

The sponsor is the sole owner of the data and is responsible of all the clinical trial activities from study design, development, data collection, management, analysis, interpretation of data, writing and the decision to submit the report for publication written by the Principal Investigator.

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